Pharmacogenetic Study of Drugs Affecting *Mycobacterium tuberculosis*

Samira Shabani, *et al*., 2024. International Journal of Mycobacteriology (IF 1.2, H-Index 29, Q4)
Presented on September 20, 2024

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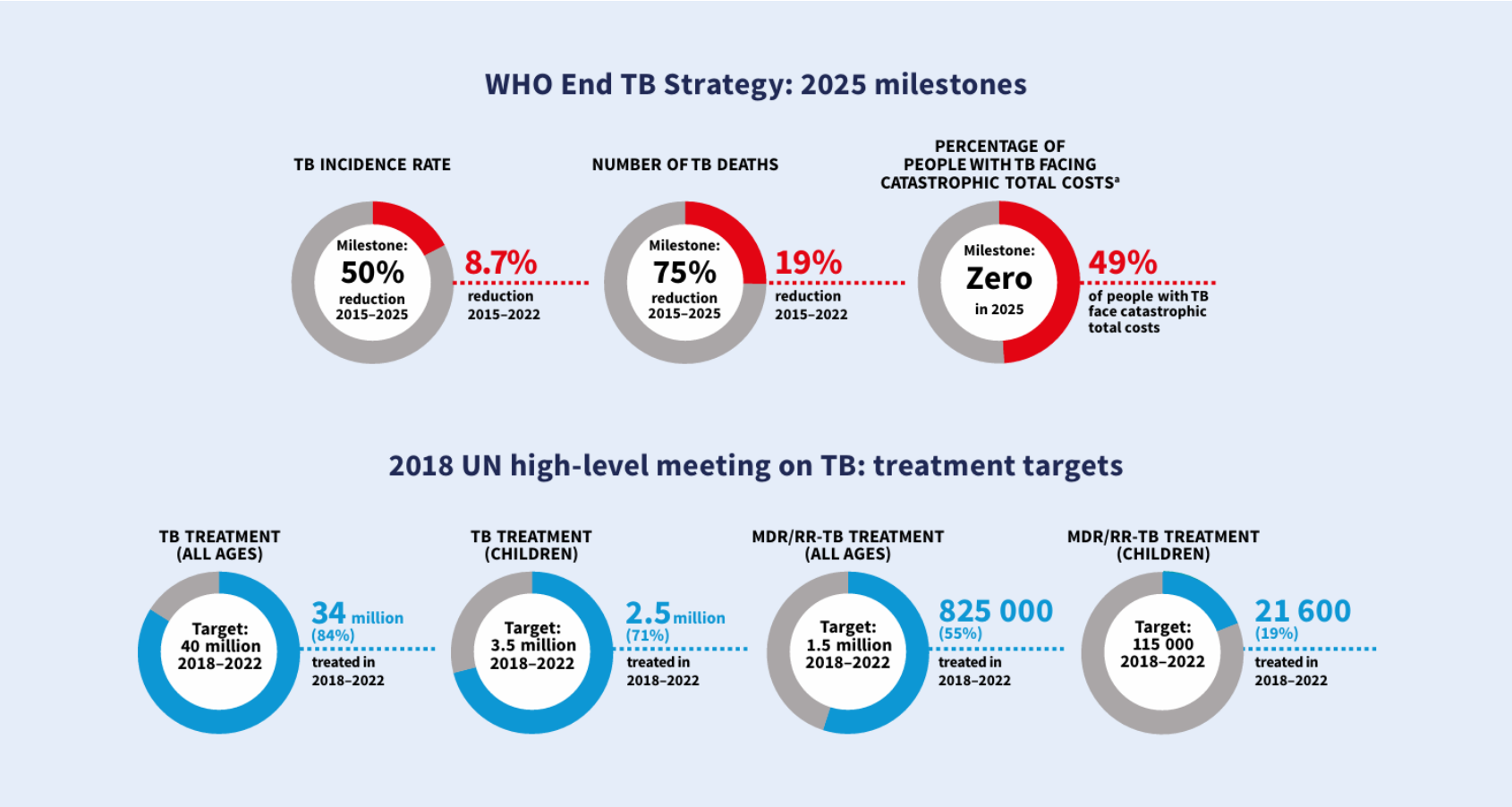
Aim of manuscript and relevance

- Tuberculosis (TB) is a preventable and usually curable disease.
- TB is caused by the bacillus *Mycobacterium tuberculosis*, which is spread when people who are sick with TB expel bacteria into the air.
- In 2022, TB was the world's second leading cause of death from a single infectious agent.

Global Tuberculosis Report (WHO, 2023)

Aim of manuscript and relevance

Therefore, is one of major problems for public health.



Global Tuberculosis Report (WHO, 2023)

Introduction

TB first-line antibiotics treatment consist of a multidrug regimen lasting 6-8 months

- Isoniazid (INH)
- Rifampicin (RIF)
- Pyrazinamide (PZA)
- Ethambutol (EMB)

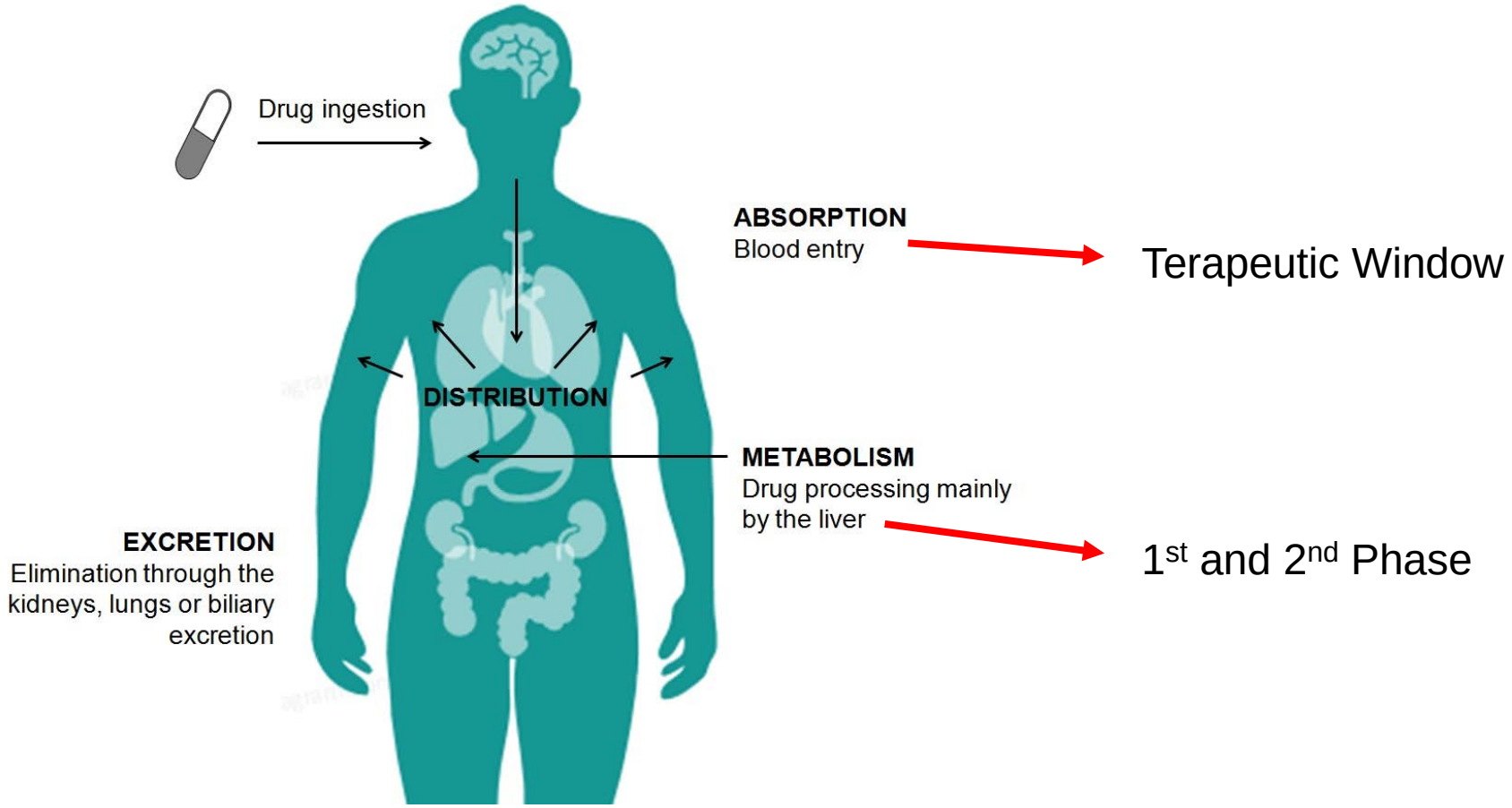
In Mexico, the NOM-006-SSA2-2013 provides the information to prevention and control of TB.

The response to these agents is not always predictable and sometimes may lead to adverse effects or non-response to treatment.

Introduction

Absorption, Distribution, Metabolism and Excretion process

However, many studies have shown that the toxicity of anti-TB drugs is related to genetic changes, affecting the achievement of maximum drug concentrations.



Introduction

The development of pharmacogenetics can bring a revolution in the field of treatment and may lead to optimal targeted therapies and modification of response to standard therapy.

Pharmacogenomics

Age

Genetic

Gender

General Health

Other medications

Lifestyle

Introduction

For metabolism of two drugs anti-TB:

Arylamine N-acetyltransferase 2 (NAT2)

- NAT2 gene
- Lead INH metabolism
- Single-nucleotide Polymorphisms (SNPs) may affect the effectiveness of enzyme

Organic anion transporter polypeptide (OATP1B1)

- SLCO1B1 gene
- Lead RIF entry to liver for metabolism
- SNPs may affect the expression of protein.

Next-generation sequencing (NGS) has emerged as a powerful tool for understanding the genetic background of various infectious diseases, including TB.

Introduction

The aim of this article is to characterize the allelic frequency of NAT2 and SLCO1B1 SNPs**,** genes that are involved in the metabolic pathway of the proposed drugs in the treatment of pulmonary TB in the Iranian population using whole-exome sequencing (WES) technique.

Materials & Methods

Ethical statement

The study was approved by the Ethics Committee of of National Institute of TB and Lung Diseases Theran, Iran.

Type of sampling

- 30 blood samples
- 2 mL of blood taken from people and stored EDTA vacutainers at −20°C
- Written consent forms collected from every patient.

DNA extraction

Omega Bio-tek Nucleic Acid Purification Kits

Spectrophotometric analyses of DNA

The concentration and purity absorbance (ratio at A^{260}/A^{280} nm) assessed with Thermo Scientific NanoDropTM 1000 Spectrophotometer

Materials & Methods

Visualizing DNA by agarose gel electrophoresis

1% agarose gel
TBE buffer
Voltage of 80 V for 60 min
Ethidium bromide
Gel Doc XR + Imaging System

WES and data analysis

Next-Generation Illumina Sequencing Illumina Novoseq6000 and Agilent SureSelect V7/Twist2 + kit.

- The 1000 Genomes Project Consortium
- Genet Med
- Database of SNP Exome Variant Server
- NHLBI Exome Sequencing Project
- Online Mendelian Inheritance in Man
- OMIM®

Materials & Methods

Statistical data analysis

The SNPStats and genotypic frequency were evaluated with SPSS software V22.0. $P < 0.05$ was considered statistically significant.

Results

Extracted DNA quality and quantity valuation

Gel electrophoresis showed a single, high molecular weight DNA band with the absence of RNA contamination.

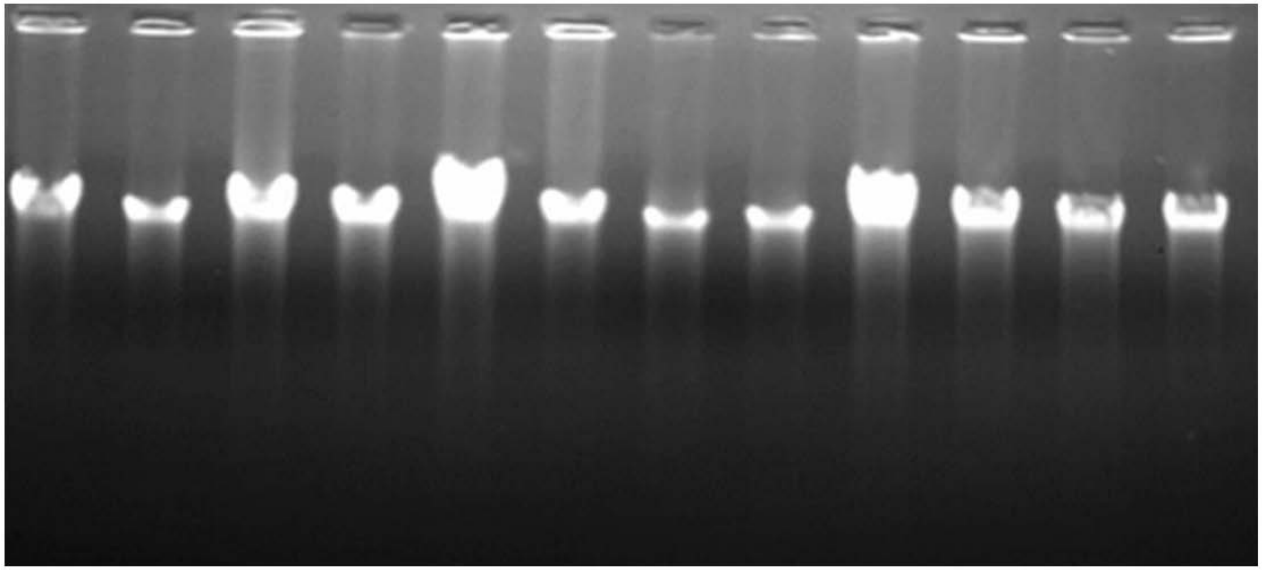


Figure 1: The genomic DNA isolated from sample in 1% agarose gel. None of the DNA samples displayed significant smearing, which shows degradation of sample

Results

The spectra range obtained of samples was between 2000 and 400 ng and totally 1000 ng concentration was considered for each reaction.

Ratio of A^{260}/A^{280} nm

The amount was evaluated in the normal range of 1.8–2, indicates the absence of protein and RNA contamination.

Ratio of A^{260}/A^{230} nm

The amount of which was evaluated in the normal range of 2–2.4, which indicates no polysaccharide contamination.

Results

NAT2 gene SNPs frequency in Iranian population

Six SNPs

Anottation
Intron
Acetylation activity
Unknown

Six SNPs

Anottation
Synonymous/Missense
Acetylation activity
Slow acetylation

One SNPs

Anottation
3´UTR
Acetylation activity
Unknown

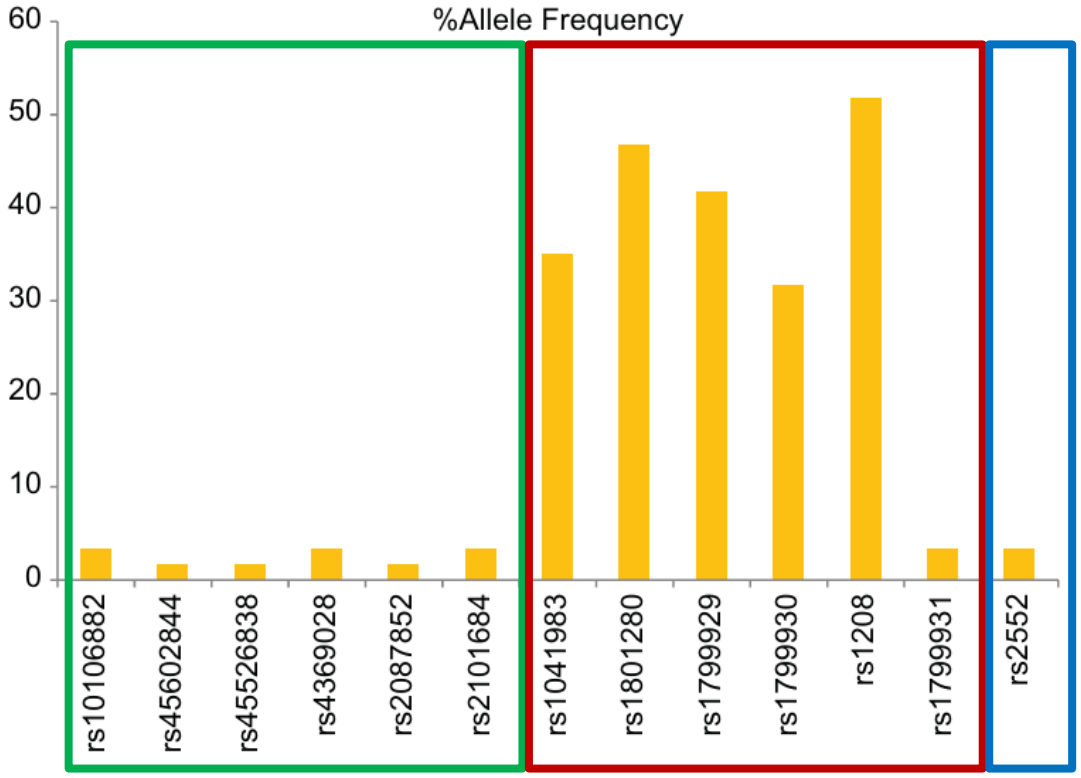


Figure 2: Schematic chart of the observed N-acetyltransferase 2 (*NAT2*) variant (locus on 8p22) in Iranian population. This chart displays the allele frequency of 13 single-nucleotide polymorphisms observed in *NAT2* gene in noncoding and coding region of exon 2 among the thirteen Iranian population samples

Results

Table 1: The observed frequency results of N-acetyltransferase 2 gene variant which can be distinguished by mutation site, mutation type, allelic frequency (mutant frequency, allele frequency, and heterozygote frequency), and enzyme activity

Variant *NAT2*	Transcript consequence	Annotation	Percentage mutant (MF)	Percentage heterozygotes	Percentage wild	Percentage AF	Acetylation activity
rs10106882	8:18077007 8:18219498 G/C	Intron	3.3 (0.0–10.0)	0.0	96.7 (90.0–100.0)	3.33	Unknown
rs45602844	8:18251510 8:18394000 G/A	Intron	0.0	3.3 (0.0–10.0)	96.7 (90.0–100.0)	1.66	Unknown
rs45526838	8:18251558 8:18394048 T/G	Intron	0.0	3.3 (0.0–10.0)	96.7 (90.0–100.0)	1.66	Unknown
rs4369028	8:18251581 8:18394071 A/G	Intron	3.3 (0.0–10.0)	0.0	96.7 (90.0–100.0)	3.33	Unknown
rs2087852	8:18251926 8:18394416 A/G	Intron	0.0	3.3 (0.0–10.0)	96.7 (90.0–100.0)	1.66	Unknown
rs2101684	8:18252000 8:18394490 G/A	Intron	3.3 (0.0–10.0)	0.0	96.7 (90.0–100.0)	3.33	Unknown
rs1041983	8:18257795 8:18400285 C/T	Synonymous	20.0 (6.7–33.3)	30.0 (16.7–46.7)	50.0 (30.0–69.9)	35	Slow
rs1801280	8:18257854 8:18400344 T/C	Missense	26.7 (13.3–43.3)	40.0 (23.3–56.7)	33.3 (16.7–50.0)	46.66	Slow
rs1799929	8:18257994 8:18400484 C/T	Synonymous	16.7 (6.7–33.3)	50.0 (33.3–66.7)	33.3 (16.7–50.0)	41.66	Slow
rs1799930	8:18258103 8:18400593 G/A	Missense	13.3 (3.3–26.7)	36.7 (20.0–53.3)	50.0 (33.3–66.7)	31.66	Slow
rs1208	8:18258316 8:18400806 G/A	Missense	33.3 (16.7–53.3)	36.7 (20.0–53.3)	30.0 (13.3–46.7)	51.66	Slow
rs1799931	8:18258370 8:18400860 G/A	Missense	0.0	6.7 (0.0–16.7)	93.3 (83.3–100.0)	3.33	Slow
rs2552	8:18258534 8:18401024 T/C	3' UTR	0.0	6.7 (0.0–16.7)	93.3 (83.3–100.0)	3.33	Unknown

MF: Mutant frequency, AF: Allele frequency, NAT2: N-acetyltransferase 2, UTR: Untranslated region

Results

SLCO1B1 gene SNPs frequency in Iranian population

Four SNPs

Anottation
Missense/Synonymus
Protein activity
Fast

One SNPs

Anottation
Missense
Protein activity
Slow activity

One SNPs

Anottation
Synonymus
Protein activity
Normal activity

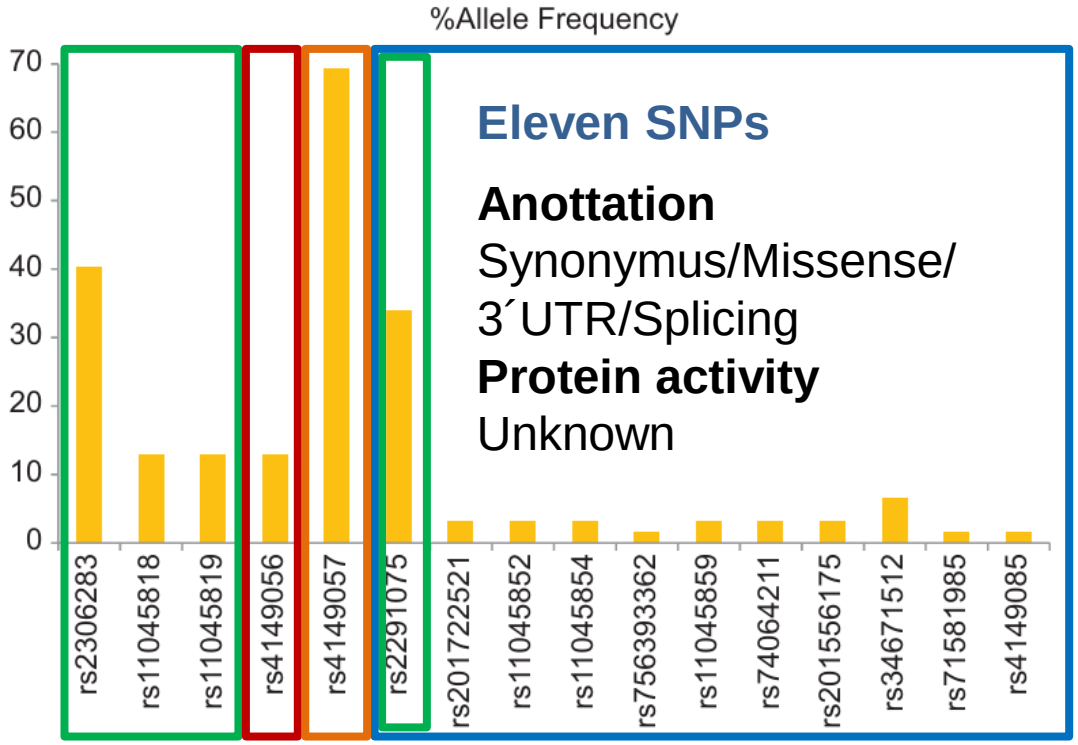


Figure 3: Schematic chart of the observed solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) variant (chromosome 12p12.1) in Iranian population. This chart displays the allele frequency of 16 single-nucleotide polymorphisms observed in *SLCO1B1* gene in coding region (15 exons) of this gene among the 30 Iranian population samples

Results

Table 2: The observed frequency results of solute carrier organic anion transporter family member 1B1 gene variant which can be distinguished by mutation site, mutation type, allelic frequency (mutant frequency, allele frequency, and heterozygote frequency), and enzyme activity

Variant *SLCO1B1*	Transcript consequence	Annotation	Percentage mutant	Percentage heterozygotes	Percentage wild	Percentage AF	Enzyme activity
rs2306283	12: 21329738 A/G 12: 21176804	Missense	22.6 (9.7–35.5)	35.5 (19.4–51.6)	41.9 (25.8–61.3)	40.32	Fast
rs11045818	12: 21329761 G/A 12: 21176827	Synonymous	0.0	25.8 (9.7–41.9)	74.2 (58.1–90.3)	12.9	Fast
rs11045819	12: 21329813 C/A 12: 21176879	Missense	0.0	25.8 (9.7–41.9)	74.2 (58.1–90.3)	12.9	Fast
rs4149056	12: 21331549 T/C 12: 21178615	Missense	3.2 (0.0–9.7)	19.4 (6.5–35.5)	77.4 (61.3–90.3)	12.9	Slow
rs4149057	12: 21331599 T/C 12: 21178665	Synonymous	51.6 (32.3–70.9)	35.5 (19.4–54.8)	12.9 (3.2–25.8)	69.35	Normal activity
rs2291075	12: 21331625 C/T 12: 21178691	Synonymous	12.9 (3.2–25.8)	41.9 (25.8–58.1)	45.2 (29.0–61.3)	33.87	Fast
rs2291075	12: 21331860 A/G 12: 21178926	Missense	0.0	6.5 (0.0–16.1)	93.5 (83.9–100.0)	3.22	Unknown
rs11045852	12: 21349885 A/G 12: 21196951	Missense	0.0	6.5 (0.0–16.1)	93.5 (83.9–100.0)	3.22	Unknown
rs11045854	12: 21350034 G/A 12: 21197100	Synonymous	0.0	6.5 (0.0–16.1)	93.5 (83.9–100.0)	3.22	Unknown
rs756393362	12: 21353607 G/A	Splicing	0.0	3.2 (0.0–9.7)	96.8 (90.3–100.0)	1.61	Unknown
rs11045859	12: 21355537 G/A 12: 21202603	Synonymous	0.0	6.5 (0.0–16.1)	93.5 (83.9–100.0)	3.22	Unknown
rs74064211	12: 21358922 C/T 12: 21205988	Synonymous	0.0	6.5 (0.0–16.1)	93.5 (83.9–100.0)	3.22	Unknown
rs201556175	12: 21377702 G/A 12: 21224768	Synonymous	0.0	6.5 (0.0–16.1)	93.5 (83.9–100.0)	3.22	Unknown
rs34671512	12: 21391976 A/C 12: 21239042	Missense	0.0	12.9 (3.2–25.8)	87.1 (74.2–96.8)	6.45	Unknown
rs71581985	12: 21392169 C/T 12: 21239235	3'UTR	0.0	3.2 (0.0–9.7)	96.8 (90.3–100.0)	1.61	Unknown
rs4149085	12: 21392290 T/C 12: 21239356	3'UTR	0.0	3.2 (0.0–9.7)	96.8 (90.3–100.0)	1.61	Unknown

AF: Allele frequency, *SLCO1B1*: Solute carrier organic anion transporter family member 1B1, UTR: Untranslated region

Discussion

13 frequent SNP of NAT2 were found. Seven of them were in exon 2 coding region.

All these polymorphisms cause slow acetylation, may lead liver toxicity, using high dosage of INH in TB treatment is not recommended.

16 frequent SNP of SLCO1B1 were found. One of them might cause a decrease in gene expression, which leads to upper levels of RIF in the bloodstream.

In both cases, investigation of genetic variation of human TB treatment is recommended.

Conclusion

- Seven frequent SNPs were identified in the NAT2 gene. These SNPs can influence the activity of the NAT2 enzyme.
- Sixteen frequent SNPs were detected in the SLCO1B1 gene. These genetic variants can impact the pharmacokinetics of RIF.
- Since genetic variations can affect the metabolism of these drugs. Their interpretation may facilitate therapeutic drug monitoring.
- Incorporating these findings into clinical practice has the potential to optimize TB therapy and enhance treatment outcomes.

Take-away, Food for thought

- How feasible is it to introduce such studies in public health care facilities?
- Are there other methods that allow such studies to be carried out?
- Role and presence of the pharmacist in the health system.

RVPVE

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